Gonadal Steroid Hormones Levels in a Bitch with True Bilateral Hermaphroditism


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Abstract: Intersexualism occurs in all species of mammals and the pathogenesis of abnormal development of the gonads is still unclear. Thus, the aim of this report is to describe the hormonal and histopathological findings from a case of bilateral true hermaphroditism in a mixed-breed bitch due to the few case reports published involving hormonal levels in dogs with intersexuality. Serum was obtained from the animal before surgery and was submitted to hormone measurements. Progesterone and testosterone were evaluated by radioimmunoassay and estrogen by ELISA (immune-enzymatic assay). The values of progesterone, testosterone and estrogen concentrations were 2.26 ng/mL, 0.05 ng/mL and 9.7 pg/mL, respectively. The proportion of ovarian tissue found in the ovotestes after histopathological examination was higher in relation to seminiferous tubules, which may explain the low level of serum testosterone. Low concentration of serum testosterone may have contributed to the partial virilization of external genitalia, since the animal only had clitoral hypertrophy. Estrogen and progesterone levels found were compatible with the initial stage of estrus (preovulatory LH (luteinizing hormone) surge), which shows that ovarian follicles found in the bilateral ovotestes contributed to the occurrence of the estrous cycle before surgery. Quantitation of serum hormones may also shed light on the cause of disturbances in sex differentiation in canine species. More studies are needed to elucidate the real cause of intersex in dogs.

Key words: Dog, sexual chromosome disorders, sex reversal, ovotestis.

1. Introduction

Disturbances in sex differentiation, also called intersex may occur in all species of mammals, and its pathogenesis is still not completely clear [1-4].

Intersex is characterized as true hermaphrodites or pseudohermaphrodites according to the histological state of the gonads and external genitalia appearance [2-5]. True hermaphroditism involves a bilateral manifestation of ovotestes, unilateral ovotestis with ovary or testis on the contralateral gonad, or a parallel manifestation of ovary and testis. Pseudohermaphroditism presented normal gonads with an incompatible genital phenotype [2]. Abnormalities of genetic or chromosomal origin [6, 7], as well as inappropriate hormonal or chemical exposure, may lead to these development disorders [4, 8].

Although there are many case reports concerning intersexuality in canine species, scarce studies evaluated the sexual hormones profile in dogs with intersex. Thus, the aim of this case report is to describe the sexual hormones concentrations and histopathological findings of the gonads from a case of true hermaphroditism in a mixed-breed bitch.

2. Case Report

A healthy mixed-breed bitch weighting 10 kg, age ranging from 1 to 3 years, based on dental evaluation, was submitted to elective ovariohysterectomy during an animal control campaign. Preoperative clinical examination of external genitalia revealed an enlarged clitoris protruded from the rima vulvae (Fig. 1A). Scrotum or testes were not detected during extra
abdominal evaluation. The owner did not provide information about estrous cycle and behavior.

Celiotomy revealed bicornuated uterus with normal cervix and bilateral gonads (Fig. 1B) recovered by ovarian bursa. The gonads measured 14 × 10 × 5 mm (left) and 13 × 8 × 5 mm (right), with a soft consistency, compact aspect, containing scarce cystic areas of yellowish colored, which were submitted to histopathological evaluation. The structures were identified as ovotestis containing numerous ovarian follicles in different developmental stages (primordial, primary, secondary and tertiary follicles) (Fig. 2A and 2C), seminiferous tubules (Fig. 2D) with no germ cells arranged at the periphery and interstitial cells (Fig. 2B). Amount of ovarian tissue found in the ovotestes was greater than testicular tissue.

Sexual hormone concentrations were assessed before ovariohysterectomy. The blood was collected by venipuncture of jugular vein with a 25.0 × 0.7 mm needle coupled to a 3.0 mL syringe to obtain the serum. The sample was centrifuged at 1,000 g for 15 min and the serum was frozen for analysis. Progesterone and testosterone levels were evaluated by radioimmunoassay using commercial kits (RIA Progesterone and RIA Testosterone, Beckman Coulter, Diagnostic Systems Laboratories, Inc., Webster, TX, USA) according to the manufacturer. The reading was performed in gamma counter (WIZARD2 Automatic Gamma Counter, Perkin Elmer, Waltham, MA, USA). Estrogen serum concentration was evaluated by ELISA (Estradiol EIA Kit, ref. 582251, Cayman Chemical Company, Ann Arbor, MI, USA) according to the manufacturer. The values of progesterone, testosterone and estrogen serum were 2.26 ng/mL, 0.05 ng/mL and 9.7 pg/mL, respectively.

Vaginal cytology was performed before surgery to evaluate the characteristic changes in exfoliated vaginal epithelial cells as a result of changing secretory patterns of estrogens. The exfoliated cells were obtained by passing a cotton-tipped swab into the caudal vagina. The swab was directed dorsocranially and rolled over the dorsal vaginal mucosa. Then it was gently rolled over a clean microscope slide, which was stained with Diff-Quik. The smear was scanned on 40× to 200× under a light microscope to determine cell morphology according to estrous cycle phase. More than 90 per cent superficial cells, low erythrocytes and neutrophils were observed in the vaginal smear.

The karyotyping had not been performed since the owner did not allow the exam.

Fig. 1 (A): Prominent clitoris (arrow) protruded from rima vulvae; (B): both gonads after ovariohysterectomy measuring 14 × 10 × 5 mm (left) and 13 × 8 × 5 mm (right).
3. Discussion

The establishment of diagnosis, intersexuality classification and the determination of both gonadal and chromosomal sex are required [9, 10]. Expression of SRY gene may also lead to ovotestes development and its diagnosis may also be necessary, which can be performed by PCR (polymerase chain reaction) from gonadal tissue [11]. Unfortunately, in the present study it was not possible to perform karyotyping and PCR to assess congenital disease, to determine if sex chromosome anomalies or gene disorders were the cause of the true hermaphroditism.

The sex chromosome anomalies include congenital changes of sex chromosome number, XX/XY chimaerism/mosaicism resulting from random events, and the presence of Y chromosome with expression of SRY gene [9-11]. However, macroscopically evaluation of the reproductive tract of the bitch showed an ambiguous and partially masculinized (clitoromegaly) external genitalia, which is typically shown in XX true hermaphrodite dogs [9, 11, 12].

Inappropriate hormonal or chemical exposure, like androgens and progestins, mainly during pregnancy also can lead to the development of sexual disorders in puppies [4, 8, 13-15]. Androgens have been used to suppress canine estrus in veterinary practice, but when administered during gestation in clinical practice the external genitalia of female offspring was masculinized. Also, testosterone and progestagens induced similar effects [16]. Although it is often stated that steroidal hormones have no effect at all on gonadal...
Rooster et al (2006) [15] described a case of true hermaphroditism in six female puppies after administration of a synthetic testosterone mixture in a pregnant bitch on approximately day 40 of gestation. The disturbance of sexual differentiation depends upon the stage of embryogenic development, kind of agent, amount and pathway of administration, and duration of its use [8, 17, 18]. Unfortunately, in our reported case we did not have previous history of the bitch attended.

The hormonal profile of bitch before ovariohysterectomy indicated a low concentration of estrogen and testosterone, and increased progesterone was compatible with the preovulatory LH surge in the estrus stage. Testosterone level (0.05 ng/mL) was below male reference ranges (0.4-6.0 ng/mL) [19], however it was similar to values observed in nonpregnant bitches during estrus after the preovulatory LH peak (< 0.05-0.1 ng/mL) [20]. The low testosterone level was compatible with the ovotestes histopathological findings of fewer testicular tissues containing no sperm cells in contrast with ovarian tissue, although the clitoral hypertrophy was observed. It has been reported that ovotestes, in cases of hermaphroditism due to incompletely clarified mechanisms, show prevalent ovarian component in contrast to testicular component [2, 21].

Despite the amount of ovarian tissue in the ovotestes, the concentration of estrogen and progesterone may vary according to the phase of the estrous cycle. At estrus, the estradiol values continue its decline from peak values to intermediate values (10-20 pg/mL) and serum progesterone rapidly increases above 1-3 ng/mL [22]. The vaginal cytology findings, and hormone concentration (estrone 9.7 pg/mL and progesterone 2.26 ng/mL) indicated that the evaluated bitch was at estrus phase of estrous cycle. In addition, progesterone can also be produced by Leydig cells [23, 24]. These findings demonstrated that the ovarian tissue found in both ovotestes was functional.

In a case report, a Yorkshire terrier bitch (78, XY), SRY positive, with disorder of sexual development showed low level of 17β estradiol (< 10 pg/mL) and increased serum testosterone concentration (6.7 ng/mL), but the gonads were testicles [25]. In other study, the hormone assay obtained before castration in a 78, XX, SRY negative Beagle with bilateral ovotestes revealed testosterone levels within the reference range for a male (1.490 pg/mL), whereas progesterone, estradiol and dihydrotestosterone levels were high (0.82 ng/mL, 12.7 pg/mL and 570 pg/mL, respectively) [24].

The elective ovariohysterectomy in this case was carried out aiming population control. Furthermore, many authors highly recommend ovariohysterectomy to prevent the development of neoplasm in gonads from intersexual animals, or as a measure for the removal of carrier animals with heritable disorders from the breeding program, once a bitch with true hermaphroditism may reproduce [3, 26, 27].

4. Conclusions

Histopathological evaluation and macroscopical visualization of the external genitalia allowed diagnosing a case of true hermaphroditism in a mixed breed bitch, although the lack of more tests as karyotyping or PCR did not allowed the classification of this intersexuality.

Vaginal cytology, estrogen and progesterone levels found were compatible with the initial stage of estrus, which shows that ovarian follicles found in the bilateral ovotestes were functional, contributing to the occurrence of the estrous cycle before surgery. Quantitation of serum hormones may also shed light on the cause of disturbances in sex differentiation in canine species. More studies are needed to elucidate the real cause of intersex in dogs.

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